# BACTERIAL TOXINS

Some bacteria cause disease by producing toxins, of which there are two general types: the exotoxins which are proteins, secreted by both gram-positive and gram-negative bacteria. In contrast, endotoxins, which are lipopolysaccharides, are not secreted, but instead are integral components of the cell walls of gram-negative bacteria.

## Endotoxins:

- These are heat-stable, lipopolysaccharide (LPS) components of the outer membranes of gram-negative but not gram-positive bacteria.
- They are released into the host's circulation following bacterial cell lysis.
- LPS consists of polysaccharide O (somatic antigen), which protrudes from the exterior cell surface, a core polysaccharide, and a lipid component called lipid A that faces the cell interior.
- The lipid A moiety is responsible for the toxicity of this molecule.

## Mechanism of action :

In humans, LPS binds to the lipid binding protein (LBP) in the serum which transfers it to CD14 on the cell membrane, which in turn transfers it to another non-anchored protein, MD2, which associates with Toll-like receptor-4 (TLR4). CD14 and TLR4 are present in several immune system cells) including macrophages and dendritic cells), triggering the signaling cascade for macrophage/endothelial cells to secrete pro-inflammatory cytokines and Nitric oxide that lead to" endotoxic shock."

- Exotoxin proteins are encoded by genes carried on plasmids or temperate bacteriophages.
- Exotoxins are susceptible to antibodies produced by the immune system, but many exotoxins are so toxic that they may be fatal to the host before the immune system has a chance to mount defenses against it.
- Enterotoxin is a protein toxin released by a microorganism in the intestine. Enterotoxins are cytotoxic and kill cells by altering the permeability of the epithelial cells of the intestinal wall. They are mostly pore forming toxins, causes the cells to die.

# Types of exotoxins

## 1. Cell surface-active

### a. Superantigens:

- Superantigens bridge the MHC class II protein on antigen presenting cells with the T cell receptor on the surface of T cells with a particular Vβ chain. Consequently, up to 20% of all T cells are activated, leading to massive secretion of proinflammatory cytokines, which produce the symptoms of toxic shock.

### b. Heat-stable enterotoxins:

- Some strains of E. coli produce heat-stable enterotoxins (ST), which are small peptides that are able to withstand heat treatment at 100oC. Different STs recognize distinct receptors on the cell surface and thereby affect different intracellular signaling pathways. For example, STa enterotoxins bind and activate membrane-bound guanylate cyclase, which leads to the intracellular accumulation of cyclic GMP and downstream effects on several signaling pathways. These events lead to the loss of electrolytes and water from intestinal cells.

## 2. Membrane damaging:

- Membrane damaging toxins exhibit hemolysin or cytolysin activity in vitro.

**a. Channel-forming toxins:**

***1. Cholesterol-dependent cytolysins:***

- Formation of pores by cholesterol-dependent cytolysins (CDC) such as the α toxin of Staphylococcus aureus requires the presence of cholesterol in the target cell .

***2. RTX toxins:***

- RTX (repeats in toxin) cytolysins can be identified by the presence of a specific tandemly-repeated nine amino acid residue sequence in the protein. The prototype RTX member is the HlyA hemolysin of E. coli. RTX is also found in Legionella pneumophila.

- **b. Enzymatica membrane-vagina**

## 3. Intracellular:

- They share a common mechanism of action involving:

(i) binding to specific receptors on the plasma membranes of sensitive cells,

(ii) internalization or translocation across the membrane barrier,

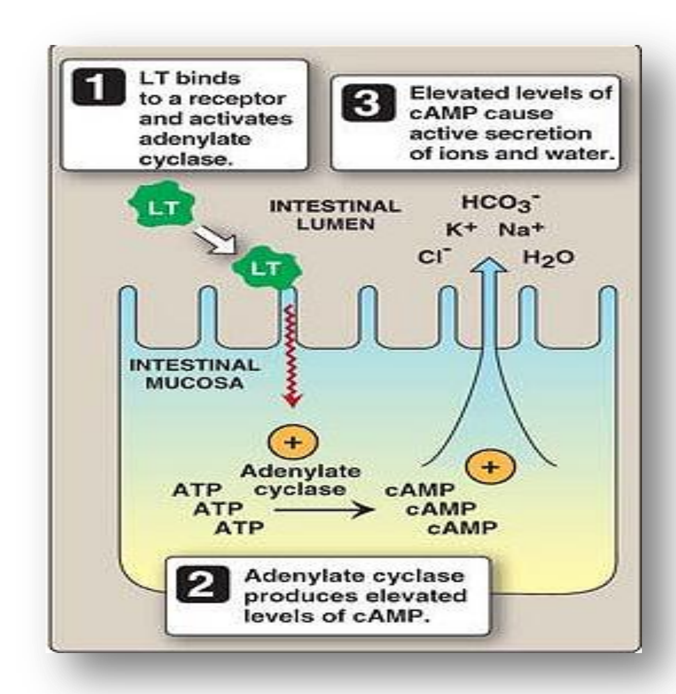
(iii) interaction with an intracellular target.

## Toxins produced by gram negative bacteria

### *E.coli*

**Enterotoxigenic E. coli (ETEC):**

ETEC are a common cause of traveler's diarrhea . ETEC produce a heat-labile enterotoxin (LT) that is similar in molecular size, sequence, antigenicity, and function to the cholera toxin (Ctx). It is a protein composed of an enzymatically active (A) subunit surrounded by 5 identical binding (B) subunits. It binds to the same identical ganglioside receptors that are recognized by the cholera toxin (i.e., GM1). ETEC may also produce a heat stable toxin (ST) that is of low molecular size and resistant to boiling for 30 minutes. ST causes an increase in cyclic GMP in host cell cytoplasm leading to the same effects as an increase in cAMP. This leads to secretion of fluid and electrolytes resulting in diarrhea.




*Action of E.coli heat labile toxin*

**Enteropathogenic E. coli (EPEC):**

EPEC are an important cause of diarrhea in infants . The EPEC attach to mucosal cells in the small intestine, causing destruction of microvilli and development of characteristic lesions. Shiga-like toxins are responsible for this destruction.

**Enterohemorrhagic E. coli (EHEC):**

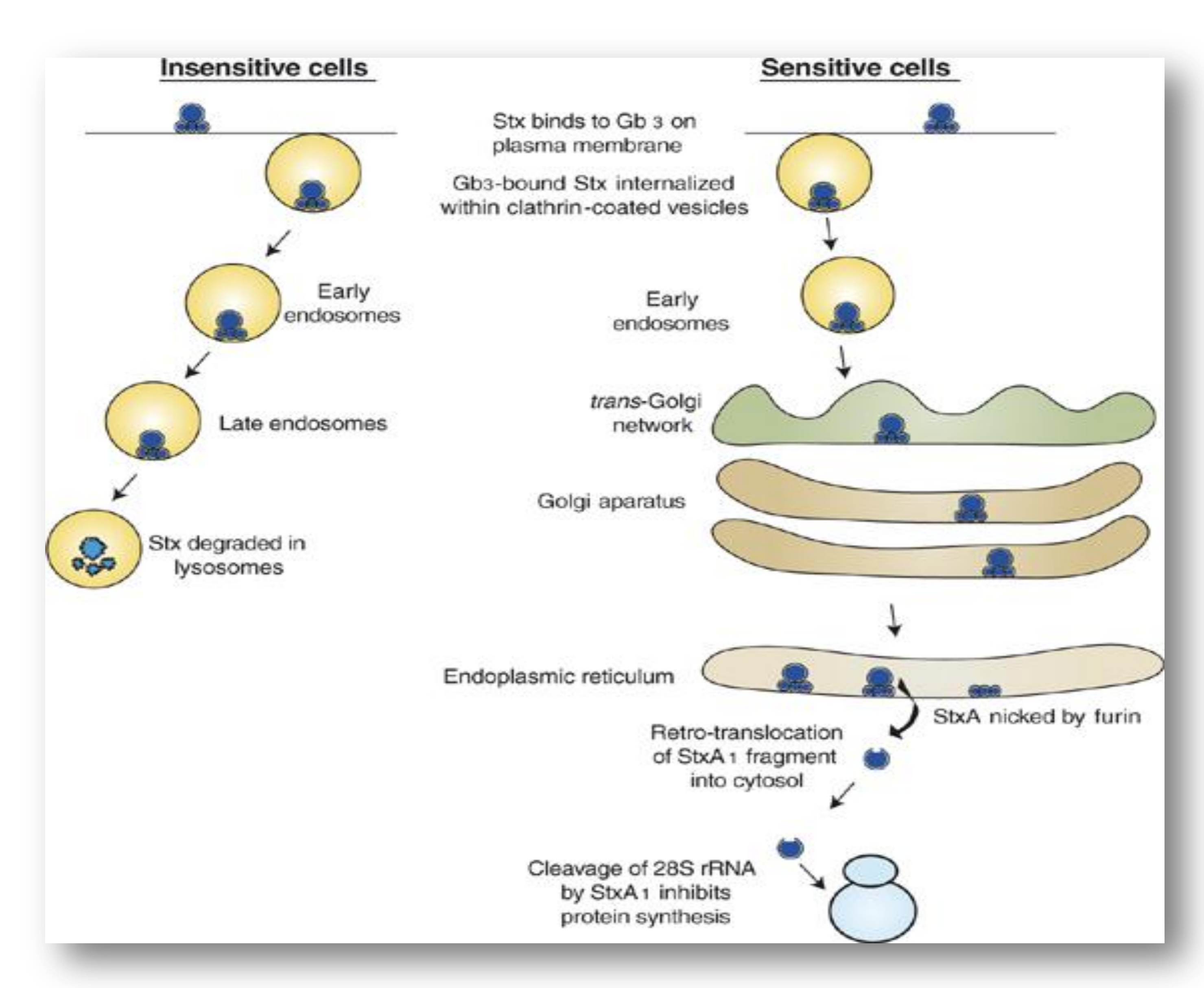
EHEC bind to cells in the large intestine, where they produce an exotoxin (verotoxin, or Shiga-like toxin), causing a severe form of copious, bloody diarrhea (hemorrhagic colitis) in the absence of mucosal invasion or inflammation. Serotype O157:H7 is the most common strain of E. coli that produces verotoxin. This strain is also associated

with outbreaks of a potentially life-threatening, acute renal failure (hemolytic uremic syndrome, HUS) .

## *Shigella*

An exotoxin (Shiga toxin) with enterotoxic and cytotoxic properties act to inhibit protein synthesis within target cells. After entering a cell, the protein functions as an N-glycosidase ،cleaving several nucleobases from the RNA that comprises the ribosome, thereby halting protein synthesis. The toxin has two subunits—designated A and B—and is one of the AB5 toxins. The B subunit is a pentamer that binds to specific glycolipids on the host cell, specifically (Gb3). Following this, the A subunit is internalised and binds to the ribosome, disrupting protein synthesis.

**NAD glycohydrolase**: *Shigella flexneri*, upon being phagocytized, produces a NAD



glycohydrolase which rapidly depletes the phagocyte of NAD, thus blocking cellular metabolism and bacterial cell killing

## *Bordetella*

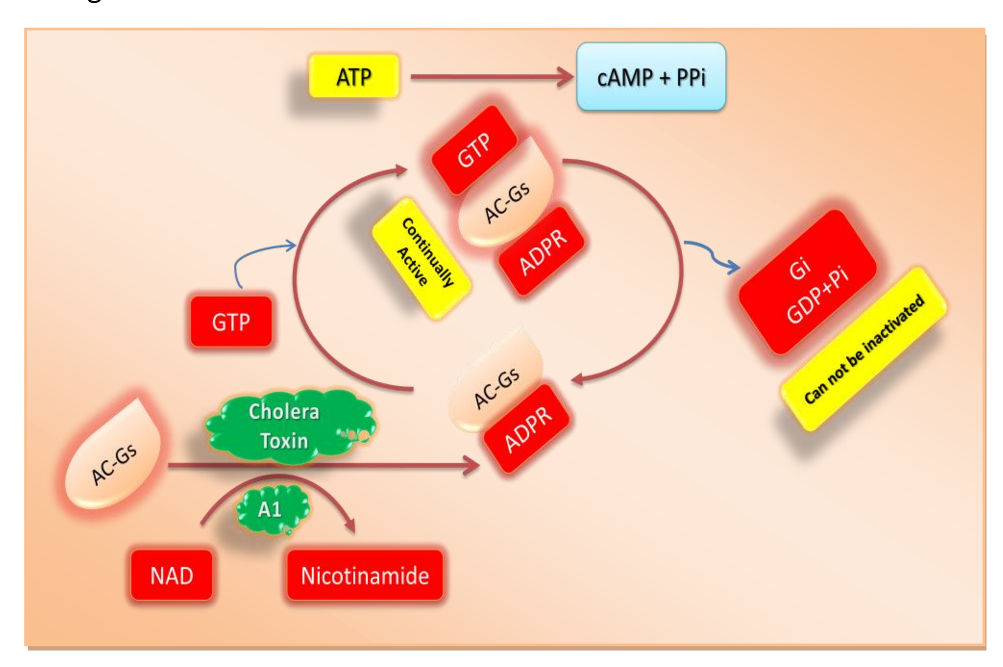
Pertussis toxin (PT) is a protein-based AB5-type exotoxin produced by the bacterium Bordetella pertussis ‹which causes whooping cough. PT is an exotoxin with six subunits . The subunits are arranged in a A-B structure: the A component is enzymatically active , while the B component is the receptor-binding portion. PT catalyzes the ADP-ribosylation of the α subunits of the G proteins making them unable to inhibit adenylyl cyclase, thus keeping levels of adenylyl cyclase and cAMP elevate.

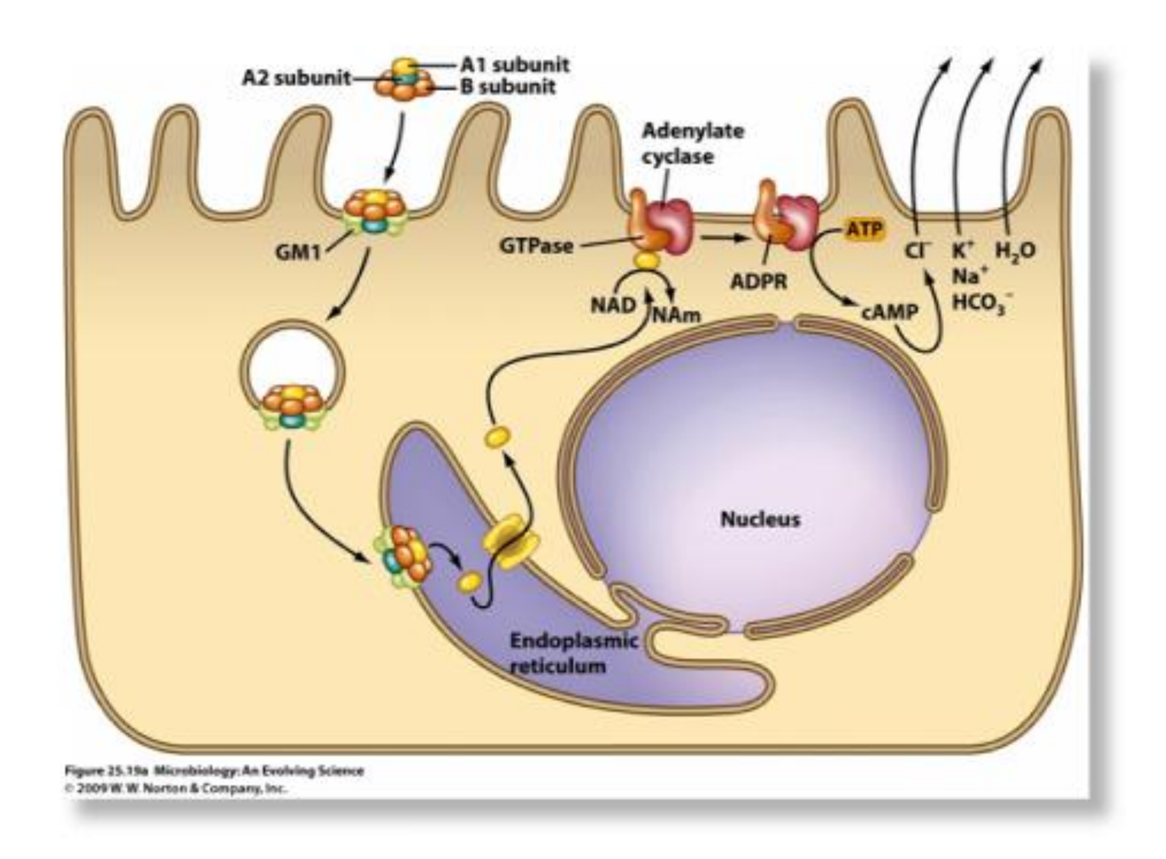
**Adenylate cyclase**: *Bordetella pertussis* produces a calmodulin-independent adenylate cyclase which inhibits and/or kills white blood cells.

## *Vibrios*

Cholera toxin is a multimeric protein composed of an A and a B subunit. The B subunit (consisting of five identical monomers) binds to the GM1 ganglioside receptor of cells lining the intestine. The A subunit has two components: A2, which facilitates penetration of the cell membrane, and A1, an ADP-ribosyl transferase that ADP-ribosylates the membrane bound Gs protein 1 Gs protein activates adenylate cyclase, which produce [illegible] his, in turn, causes an out flowing of ions and water to the lumen of the intestine.

**Mechanism of action of shiga toxin**






## *Pseudomonas*

Toxin A, the most toxic known extracellular protein of P aeruginosa, inhibit protein synthesis in susceptible cells. It achieves this by catalyzing the transfer of the ADP-ribosyl moiety of nicotinamide adenine dinucleotide (NAD) onto elongation factor 2 (EF-2) according to the following reaction:

**NAD + EF-2 ADP-ribosyle-EF-2 + Nicotinamide + $H^+$**

Some strains of P. aeruginosa produce another enzyme which appears to ADP-ribosylate eucaryotic proteins . This enzyme, termed exoenzyme S, is distinct from Pseudomonas exotoxin . Little more is known about the enzymology of exoenzyme S. Although there was an initial hint that one target cellular protein was EF-1.

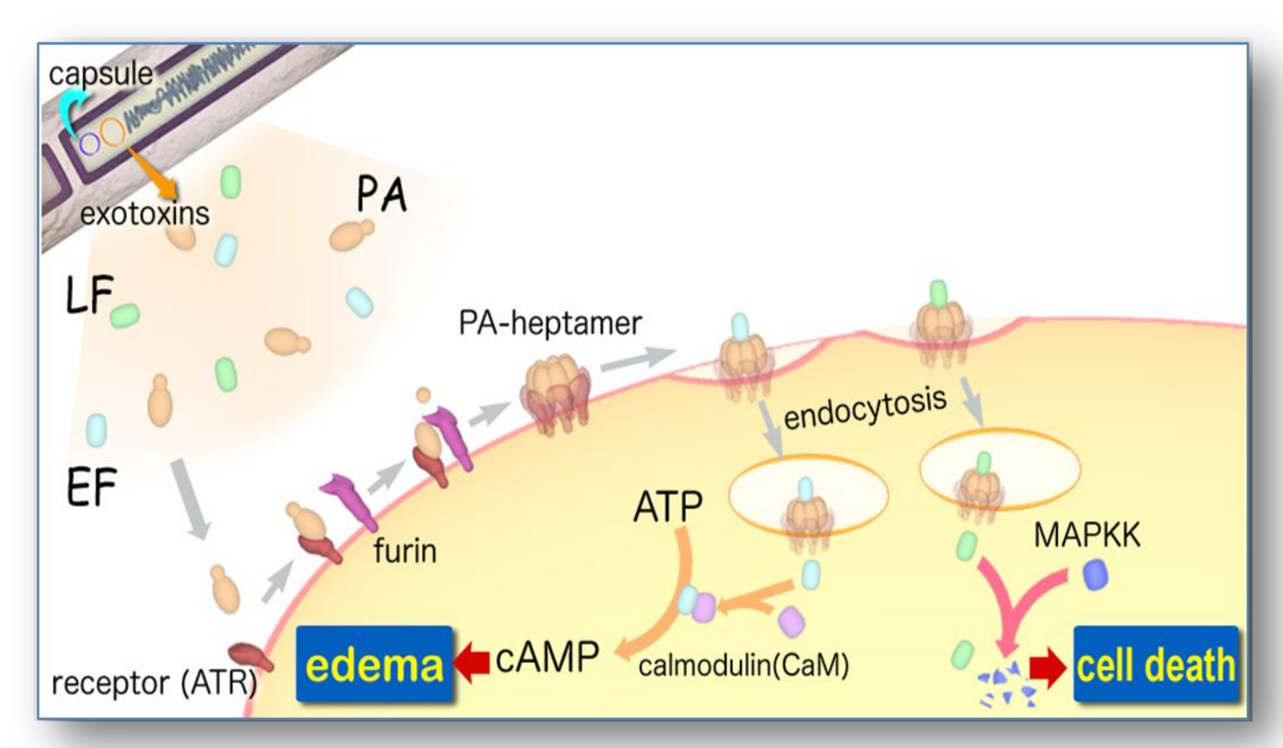
## Toxins produced by gram positive bacteria

### *Bacillus anthracis*

Anthrax toxin refers to three proteins secreted by virulent strains of the bacterium Bacillus anthracis. These three proteins act together in a synergistic way in which they are endocytosed and translocated into the cytoplasm of a macrophage, where it disrupts cellular signaling and induces cell death, allowing the bacteria to evade the immune system. This toxin is composed of three proteins:

1. The protective antigen (PA),
2. The edema factor (EF)
3. The lethal factor (LF).

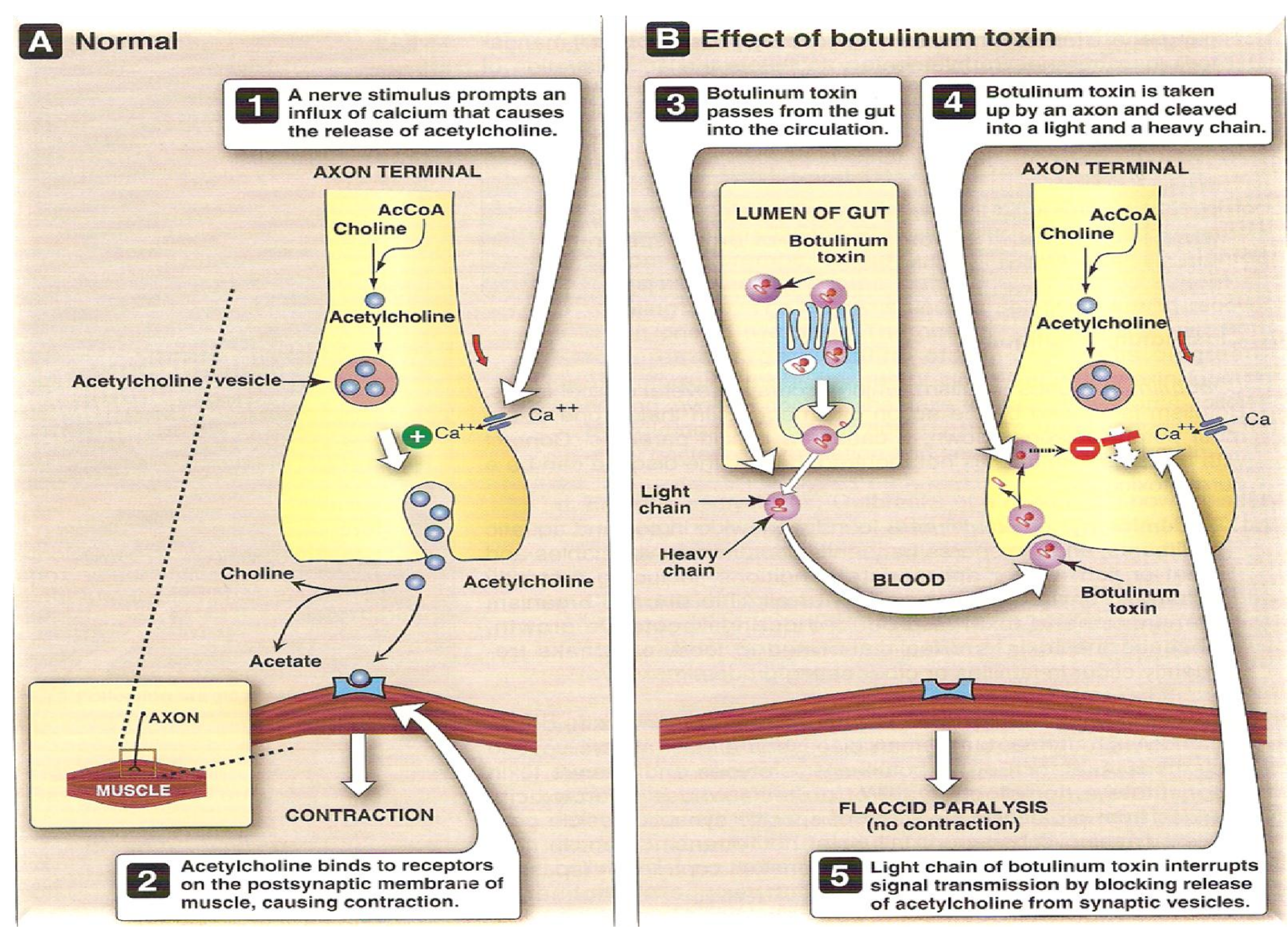
The three proteins of the anthrax toxin depend on each other for their toxic effect. Each protein is nontoxic on its own, but when combined, these proteins produce the lethal symptoms of anthrax. PA helps to shuttle EF and LF into the cell. EF acts as a Ca2+ and calmodulin dependent adenylate cyclase that greatly increases the level of cAMP in the cell. This increase in cAMP upsets water homeostasis, and impairs macrophage function . LF also helps the bacteria evade the immune system through killing macrophages. Once in these cells, LF acts as a Zn2+-dependent endoprotease that snips off the N-terminus of mitogen-activated protein kinase kinases (MAPKK). This inhibits these kinases by not allowing them to efficiently bind to their



substrates, which leads to altered signaling pathways and ultimately to apoptosis. Actions of the secreted anthrax toxins.

## *Clostridium botulinum*

Botulinum toxin is a medication and a neurotoxic protein produced by the bacterium Clostridium botulinum. It is the most toxic protein known. There are several types of botulinum toxin, designated A through G, but human disease is almost always caused by types A, B, or E. The botulinum and tetanus toxin constitute a set of proteins whose neurotoxicity arises from proteolytic cleavage of specific synaptic vesicle peptides, causing subsequent failure of neurotransmission. Botulinum toxins affect peripheral cholinergic synapses by blocking the neuromuscular junction and




inhibiting release of the neurotransmitter, acetylcholine, preventing contraction and causing flaccid paralysis.

## Clostridium difficile

C. difficile is a minor component of the normal flora of the large intestine. When antimicrobial treatment suppresses more predominant species in this community, C. difficile proliferates. Pathogenic strains produce two toxic polypeptides, designated A and B. Toxin A is an enterotoxin that causes excessive fluid secretion, but also stimulates an inflammatory response, and has some cytopathic effect in tissue culture. Toxin B is a cytotoxin; in tissue culture, it disrupts protein synthesis and causes disorganization of the cytoskeleton.

## Clostridium perfringens

Alpha toxin is a lecithinase (phospholipase C) that degrades lecithin in mammalian cell membranes, causing lysis of endothelial cells, as well as erythrocytes, leukocytes, and platelets. C. perfringens enterotoxin, a small, heat-labile protein, acts in the lower portion of the small intestine. The molecule binds to receptors on the epithelial cell surface and alters the cell membrane, disrupting ion transport (primarily in the ileum) and leading to loss of fluid and intracellular proteins. Degradative enzymes: are a variety of hydrolytic enzymes, including proteases, DNases, hyaluronidase, and collagenases, which liquefy tissue and promote the spread of infection.

## Clostridium tetani

Tetanospasmin is the neurotoxin produced by the vegetative spore of Clostridium tetani in anaerobic conditions, causing tetanus. The peptide tetanospasmin is made up of two parts: heavy or B-chain and a light or A-chain. The chains are connected by a disulfide bond. The B-chain binds to disialogangliosides (GD2 and GD1b) on the neurone membrane. The A-chain, a zinc endopeptidase, attacks the vesicle-associated membrane protein (VAMP). The action of the A-chain stops the affected neurons from releasing the inhibitory neurotransmitters GABA (gamma-aminobutyric acid) and glycine by

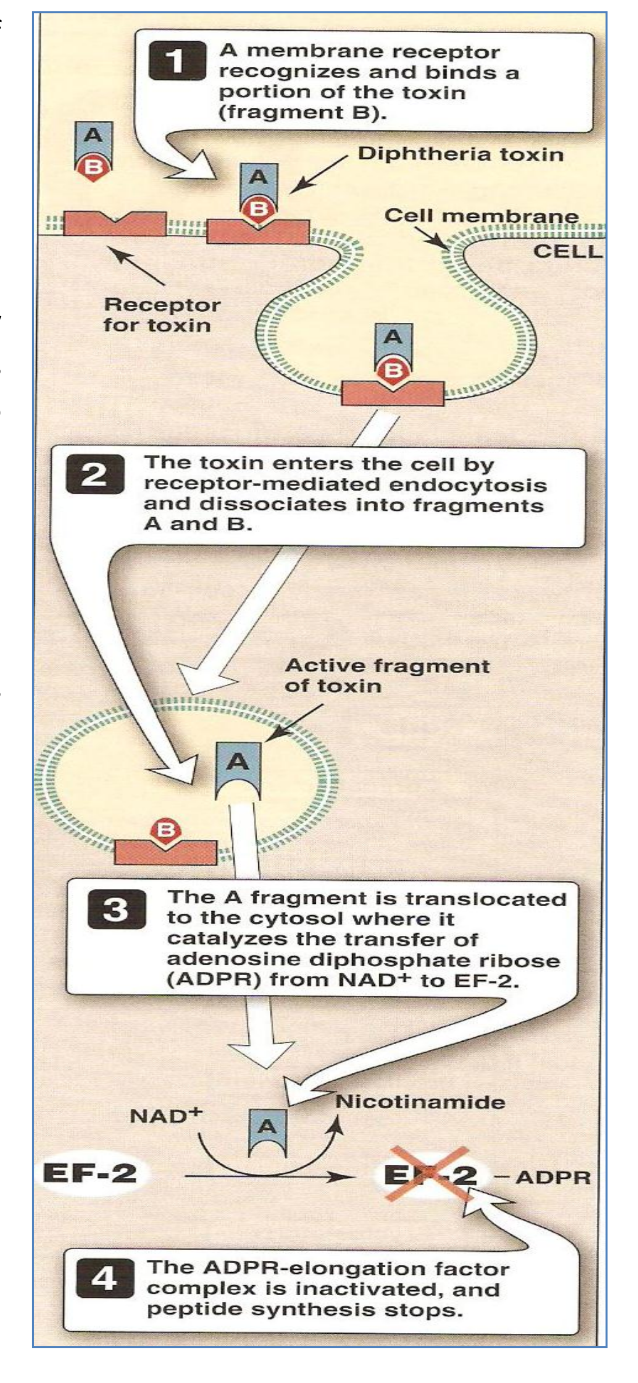
degrading the protein synaptobrevin. The failure of inhibition of motor reflexes by sensory stimulation causes generalized contractions of the agonist and antagonist musculature, termed a tetanic spasm.

### *Corynebacterium diphtheriae*

Diphtheria toxin is an exotoxin secreted by Corynebacterium diphtheriae. The toxin molecule is a heat-labile polypeptide that is composed of two fragments, A and B.

Fragment B binds to susceptible cell membranes and mediates the delivery of fragment A to its target. Inside the cell,

fragment A separates from fragment B, and catalyzes a reaction between nicotine adenine dinucleotide (NAD+) and the eukaryotic polypeptide chain elongation factor, EF-2.




### *Listeria monocytogenes*

Listeriolysin O (LLO) is a hemolysin produced by the bacterium Listeria monocytogenes. Listeriolysin O is a thiol-activated cholesterol-dependent pore forming toxin protein. After LLO lyses the phagosome, the bacterium escapes into the cytosol, where it can grow intracellularly. LLO permits L. monocytogenes to escape from phagosomes into the cytosol without damaging the plasma membrane

of the infected cell. This allows the bacteria to live intracellularly, where they are protected from extracellular immune system factors such as the complement system and antibodies.

## *Staphylococcus aureus*

**Cytolytic exotoxins**

α, β, γ, and δ toxins attack mammalian cell (including red blood cell) membranes, and are often referred to as hemolysins.

Staphylococcus aureus alpha toxin polymerizes into tubes that pierce membranes, resulting in the loss of important molecules and, eventually, in osmotic lysis. It is a membrane disrupting toxin that creates pores causing hemolysis and tissue damage. It has phospholipase C activity. Staphylococcus aureus beta toxin is a form of sphingomyelinase.

## *Staphylococcus aureus* Superantigen

**Staphylococcal Enterotoxins**

Enterotoxins are superantigens that are even more heat-stable than S. aureus; therefore, organisms are not always recovered from incriminated food. They polymerize into tubes that pierce membranes, resulting in the loss of important molecules and, eventually, in osmotic lysis . They stimulate the vomiting center in the brain by binding to neural receptors in the upper gastrointestinal (GI) tract .

**Exfoliatin**

(exfoliative toxin, ET) is also a superantigen . It causes scalded skin syndrome in children .

**Toxic shock syndrome toxin**

Toxic Shock Syndrome Toxin (TSST) is a superantigen produced by Staphylococcus aureus . It causes toxic shock syndrome by stimulating the release of large amounts of interleukin-1, interleukin-2 and tumour necrosis factor. In general, the toxin is not produced by bacteria growing in the blood; rather, it is produced at the local site of an infection, and then enters the blood stream. It cross links the TCR peptide chain with the peptide chain of the MHC-class II molecule. Because of similarities in molecular structure, it is sometimes referred to as staphylococcal enterotoxin F (SEF), although it does not cause food poisoning when ingested.

EUKARYOTIC CELL
Fibronectin
IgG
Staphylococcus

**PROTEIN A**
- Protein A binds to the Fc moiety of IgG, exerting an antiphagocytic effect.

**FIBRONECTIN-BINDING PROTEIN**
- Fibronectin-binding proteins promote binding to mucosal cells and tissue matrices.

Cytolytic exotoxins
Superantigen
Antigen-presenting cell
T cell
T
Cytokines
IL-2
IFN-γ
TNF-α

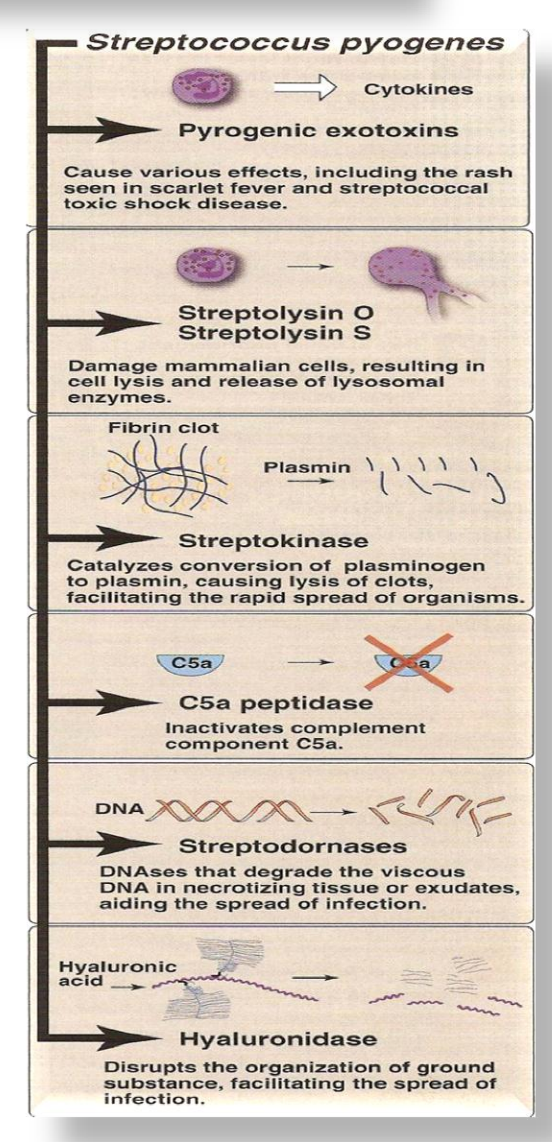
**CYTOLYTIC EXOTOXINS**
- Exotoxins attack mammalian cell (including red blood cell) membranes and are often referred to as hemolysins.

**ENZYMES**
- Coagulase
- Catalase
- Hyaluronidase
- Fibrinolysin

**SLIME PRODUCTION**
(particularly *S. Epidermitis*)

**SUPERANTIGEN EXOTOXINS**
- Toxins have an affinity for the T cell receptor–MHC Class II antigen complex.
- Toxins stimulate an enhanced T lymphocyte response.
- T cell activation can cause toxic shock by release of large amounts of T cell cytokines.

## *Streptococcus pyogenes*

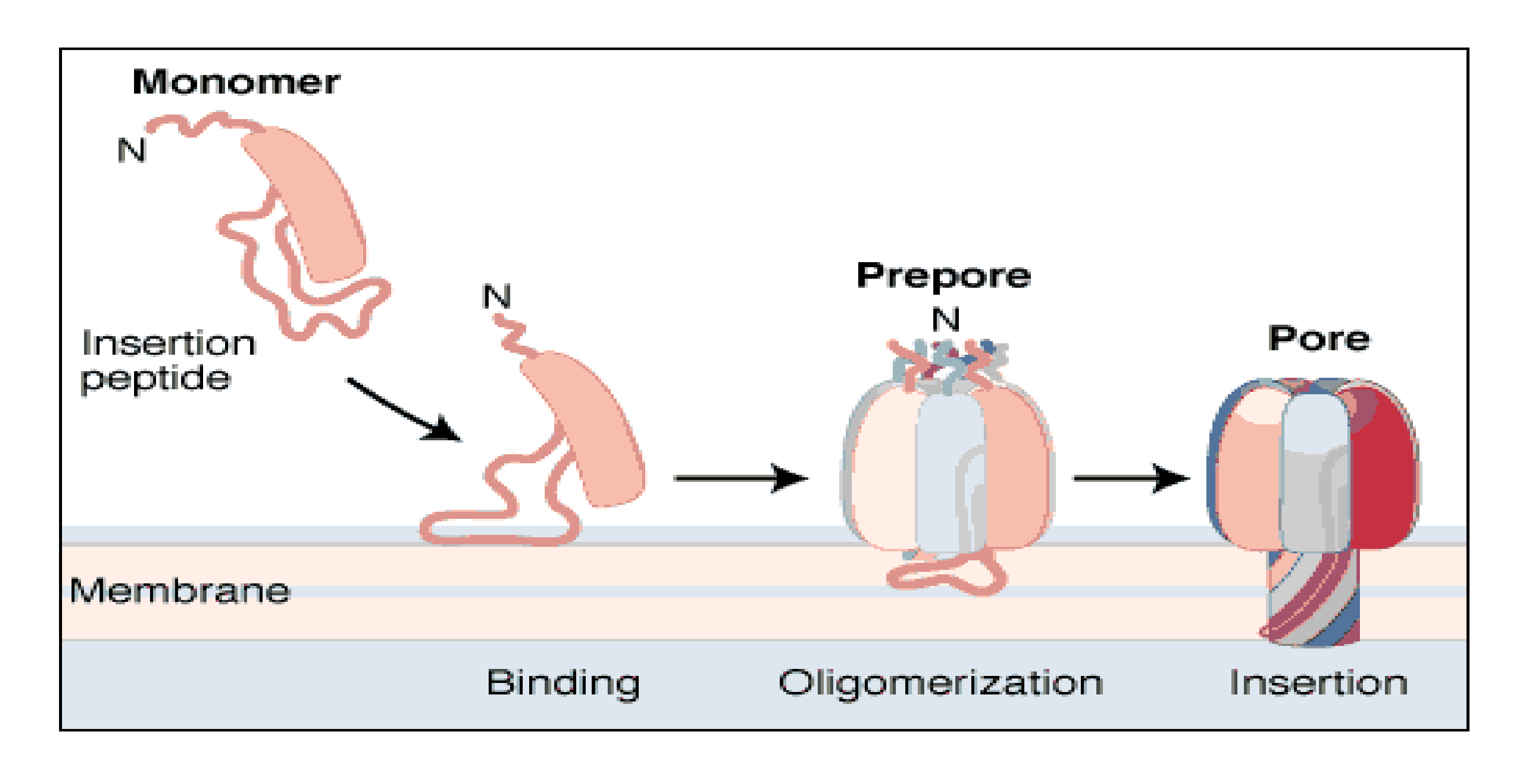



**<u>Streptolysin O & S</u>**

These are toxins which are the basis of the organism's beta-hemolytic property. Streptolysin O is a potent cell poison affecting many types of cell including neutrophils, platelets, and sub-cellular organelles. It causes an immune response and detection of antibodies to it; antistreptolysin O (ASO) can be clinically used to confirm a recent infection. Streptolysin O is cardiotoxic.

**<u>Streptococcal pyogenic exotoxins (Spe) A and C</u> :** SpeA and SpeC are superantigens secreted by many strains of S. pyogenes. These pyogenic exotoxins are responsible for the rash of scarlet fever and many of the symptoms of streptococcal toxic shock syndrome.

**<u>Streptokinase</u>** : Enzymatically activates plasminogen, a proteolytic enzyme, into plasmin which in turn digests fibrin and other proteins.

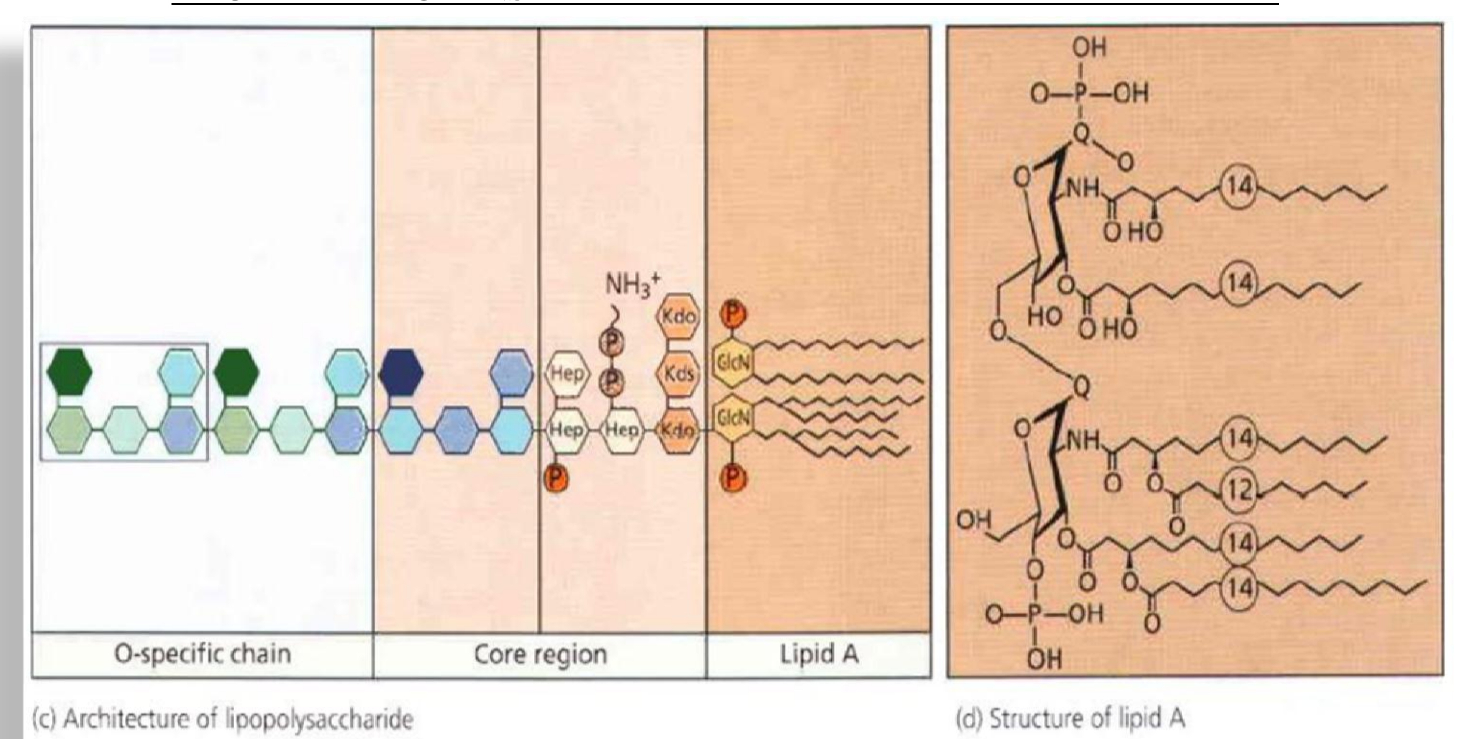



| Toxin | Bacterial source | Target | Disease |
|---|---|---|---|
| **Perfringiolysin O** | *Clostridium perfringens* | **cholesterol** | gas gangrene |
| **Hemolysin** | *Escherichia coli* | **cell membrane** | UTI |
| **Listeriolysin** | *Listeria monocytogenes* | **cholesterol** | meningitis |
| **Anthrax EF** | *Bacillus anthracis* | **cell membrane** | (edema) |
| **Alpha toxin** | *Staphylococcus aureus* | **cell membrane** | abcesses |
| **Pneumolysin** | *Streptococcus pneumoniae* | **cholesterol** | pneumonia; otitis |
| **Streptolysin O** | *Streptococcus pyogenes* | **cholesterol** | strep throat |
| **Leukocidin** | *Staphylococcus aureus* | **phagocyte membrane** | pyogenic inf. |

# INTERVENTION IN THE INTOXICATION PROCESS

In principle, antitoxins can target one or more stages in the intoxication process;

1. By preventing binding of the toxin to cell membrane receptors.
2. By preventing translocation of the toxin across the cell membrane.
3. By blocking the interaction of the toxin with the target molecule.
4. By modifying the activity of the toxin towards the target




(c) Architecture of lipopolysaccharide

(d) Structure of lipid A

## Structure and function of LPS It composed of :

1. O antigen or (O polysaccharide) 2. Core polysaccharide 3. Lipid A

**Lipid A**

Lipid A is normally a phosphorylated glucosamine disaccharide decorated with multiple fatty acids. These hydrophobic fatty acid chains anchor the LPS into the bacterial membrane and the rest of the LPS projects from the cell surface. The lipid A domain is responsible for much of the toxicity of Gram-negative bacteria. When bacterial cells are lysed by the immune system, fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhea, and possible fatal endotoxic shock (also called septic shock).

**Core**

The Core domain always contains an oligosaccharide component which attaches directly to lipid A and commonly contains sugars such as heptose and 3-deoxy-D-mannooctulosonic Acid (also known as KDO, keto-deoxyoctulosonate). The LPS Cores of many bacteria also contain non-carbohydrate components, such as phosphate, amino acids, and ethanolamine substitutents.

**O-antigen**

When LPS contains a repetitive glycan polymer this is referred to as the O antigen, O polysaccharide, or O side chain of the bacteria. The O antigen is

attached to the core oligosaccharide, and comprises the outermost domain of the LPS molecule. The presence or absence of O chains determine whether the LPS is considered rough or smooth. Full length O-chains would render the LPS smooth while the absence or reduction of O-chains would make the LPS rough. Bacteria with rough LPS usually have more penetrable cell membranes to hydrophobic antibiotics since a rough LPS is more hydrophobic. O antigen is exposed on the very outer surface of the bacterial cell, and as a consequence, is a target for recognition by host antibodies.

## LPS and virulence

Lipid A (the toxic component) and the polysaccharide side chains (the nontoxic but immunogenic components) of LPS both contribute to the virulence of gram-negative bacteria. Loss of the O-antigen increases susceptibility to complement-mediated serum killing. This is because the C5b-9 membrane attack complex (MAC) can much more readily access the bacterial cell membrane of rough rather than smooth bacteria, since in the latter the MAC forms too far from the membrane to damage the cell. Rough strains are also generally more readily phagocytosed than smooth strains. Furthermore, the Opolysaccharide is also the basis of antigenic variation among many gram-negative pathogens, including E. coli, Salmonella, Pseudomonas, and Vibrio The existence of multiple serotypes of the same organism offers opportunities to bypass the acquired immune response to a specific serotype. However, although the O-polysaccharides are highly antigenic, they seldom elicit immune responses that confer full protection to the host against secondary challenge.

## Mechanism

The pathophysiological effects of endotoxin are mediated via lipid A. LPS, released into the bloodstream by gram-negative bacteria cells undergoing lysis, is first bound by LPS-binding plasma proteins. These interact with CD14 receptors on monocytes and macrophages and subsequently lead to the induction of inflammation, intravascular coagulation, hemorrhage, and shock through the stimulation of cytokines including IL-1, IL- 6, IL-8, TNFu, and platelet-activating factor. These in turn stimulate production of prostaglandins and leukotrienes, which are powerful mediators of inflammation and the septic shock that accompanies endotoxinemia and activate both the complement and coagulation cascades. LPS can also function as a mitogen, stimulating the polyclonal differentiation and multiplication of B cells and the secretion of immunoglobulins, such as IgG and IgM.